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ORIGINAL ARTICLE – COLORECTAL CANCER

Significance of Herpesvirus Entry Mediator Expression in Human Colorectal Liver Metastasis

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ABSTRACT

Background. Herpesvirus entry mediator (HVEM) has been suggested to play various roles in cancer biology. The authors report that HVEM expression in tumor cells is associated with a reduction in the number of tumor-infiltrating lymphocytes and a poor prognosis after surgical resection in various human gastrointestinal cancers. This study aimed to clarify the clinical significance of HVEM expression in human colorectal liver metastasis (CRLM).

Methods. This study examined the cases of 104 patients with CRLM who underwent curative liver resection at Nara Medical University between 2000 and 2014. The median follow-up period was 50.2 months. Immunohistochemical staining was performed using antibodies against HVEM, CD4, CD8, and CD45RO.

Results. High HVEM expression was observed in 49 patients (47.1%) with CRLM. Expression of HVEM was not associated with age, gender, administration of preoperative chemotherapy, tumor size, number of tumors, or histologic differentiation. The high-HVEM group exhibited significantly worse overall survival (OS) than the low-HVEM group ($P = 0.002$). Multivariate analysis showed that high HVEM expression in CRLM, age of 70 years or older, and having five or more tumors are independent poor prognostic factors for OS (hazard ratio [HR], 3.35; 95% confidence interval [CI], 1.41–7.93; $P = 0.006$). The number of tumor-infiltrating CD8+ and CD45RO+ T cells was significantly lower in the high-HVEM group than in the low-HVEM group. High HVEM expression in primary colorectal cancer was significantly associated with synchronous CRLM, but not with metachronous CRLM.

Conclusions. Tumor HVEM expression might play a critical role in CRLM.

Colorectal cancer (CRC) is the third most common cause of cancer-related death, and the number of patients with CRC is increasing worldwide.[1] Colorectal liver metastases (CRLM) have also become more common, which has had a marked effect on the prognosis of CRC.[2–4]

Liver resection represents the only chance of a cure for patients with CRLM. Despite improvements in surgical techniques as well as the introduction of new chemotherapy regimens and molecular-targeted therapy, the 5-year survival rates for patients with CRLM after hepatic resection reportedly range from 33 to 61%. Therefore, novel approaches still need to be developed to improve the prognosis of patients with CRLM.

One potentially promising strategy is immunotherapy. Tumor-infiltrating lymphocytes (TILs) are considered to contribute to primary host immune responses against several types of malignant tumors.[5,6] Regarding CRC, reports associate the presence of T cell infiltrates in primary colon tumors with better overall survival (OS).[7,8] Furthermore, an association between an increased number of TILs in CRLM and improved OS has been reported.[9,10] However, because tumors have a variety of mechanisms for evading immune responses, the clinical efficacy of immunotherapy against CRC is very limited.[11,12]

Recently, immunotherapy has changed markedly with the introduction of checkpoint inhibitors such as anti-programmed cell death 1 (PD-1) and anti-programmed cell death ligand 1 (PD-L1) agents.[13] Regarding CRC, microsatellite instability-high CRC appeared to respond to checkpoint blockade with anti-PD-1 or anti-PD-L1

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agents.[12] However, microsatellite-stable CRC was much less responsive to anti-PD-1 and anti-PD-L1 agents. Furthermore, anti-PD-1 antibodies exhibited very limited antitumor activity in patients with advanced colorectal carcinoma.[14] Thus, further immunotherapy agents are needed to improve the prognosis of patients with CRC.

Herpesvirus entry mediator (HVEM), also known as tumor necrosis factor receptor superfamily 14 (TNFRSF14), was identified as a cellular mediator of herpes simplex virus entry.[15] It is expressed on several types of cells including T cells, B cells, natural killer cells, dendritic cells, and myeloid cells, as well as in non-lymphoid organs including the lungs, liver, and kidneys.[15,16]

Ligands of HVEM belong to two distinct families: TNF-related cytokines [e.g., lymphotoxin-related inducible ligand that competes for glycoprotein D binding to herpesvirus entry mediator on T cells (LIGHT) and lymphotoxin-α] and immunoglobulin (Ig)-related membrane proteins (e.g., B and T lymphocyte attenuator (BTLA) and cluster of differentiation 160 [CD160]).[17,18] Previous studies have reported that the HVEM pathway plays roles in several types of disease, including autoimmune disease, infections, and inflammation.[19,20]

Recently, HVEM has been suggested to play various roles in cancer biology.[21,22] We have reported that HVEM expression in tumor cells is associated with reductions in the number of TILs and a poor prognosis after surgical resection in human esophageal squamous cell carcinoma, hepatocellular carcinoma, and primary colorectal cancer.[23–25] However, the significance of HVEM expression in human CRLM still is largely unknown. Furthermore, the association between HVEM expression in primary CRC and CRLM has not been studied to date. This study aimed to clarify the clinical significance of HVEM expression in human CRLM.

METHODS

Patients

We examined the cases of 104 patients with CRLM who underwent curative liver resection at the Department of Surgery of Nara Medical University between 2000 and 2014. The patients were followed up until death or June 2018. The median follow-up period was 50.2 months (range, 5.5–121.4 months). The clinicopathologic stage was classified according to the International Union Against Cancer system. The remainder of each specimen was fixed in 10% phosphate-buffered formalin and embedded in paraffin.

Written informed consent was obtained from all the patients before treatment, according to our institutional guidelines. The study protocol was approved by the institutional review board (approval no. 1531).

Immunohistochemistry

Formalin-fixed, paraffin-embedded CRLM tissue samples were cut into 5-μm sections, deparaffinized, and rehydrated in a graded series of ethanol. Antigen retrieval was performed by heating the tissue sections using a target retrieval solution (pH 9.0) (DAKO, Tokyo, Japan). To block endogenous peroxidase activity, the sections were immersed in a 3% solution of hydrogen peroxide in absolute methanol for 5 min at room temperature before being washed three times in fresh phosphate-buffered saline (PBS), for 5 min each time. Then, the sections were incubated overnight at 4 °C with anti-human HVEM/TNFRSF14 antibody (MAB3561, monoclonal mouse, R&D Systems, Minneapolis, USA) diluted 1:20 with antibody diluent (DAKO) or anti-human CD45RO (UHL1, monoclonal mouse; DAKO), anti-human CD4 (1:40) (4B12, monoclonal mouse; DAKO), and anti-human CD8 (C8/144B, monoclonal mouse; DAKO) antibodies. The sections were washed three times in PB before they were incubated with the EnVision detection system (DAKO), according to the manufacturer's instructions. The sections then were counterstained with hematoxylin, dehydrated in ethanol, cleared in xylene, and coverslipped.

Evaluation of Immunostaining

The immunohistochemical staining of HVEM was evaluated according to the intensity of the staining and the percentage of positively stained tumor cells in a blinded manner. Five fields were randomly selected and evaluated by authorized pathologists who had no knowledge of the patients' clinical status or outcomes. At least 1000 tumor cells were scored in each sample, and the percentage of tumor cells positively stained was recorded as well as the staining intensity. The staining intensity was classified into the following four groups: none (0 points), weak (1 point), intermediate (2 points), and strong (3 points). The percentage of positively stained tumor cells was classified into four groups as follows: 0–25% (1 point), 26–50% (2 points), 51–75% (3 points), and 76–100% (4 points). We then evaluated HVEM expression in each tissue according to the total score by adding the scores for each parameter together (total score, 1–7). Specimens with total scores of 1–5 were classified as having low HVEM expression, and those with total scores of 6–7 were classified as having high HVEM expression. Immunohistochemical staining of CD4+, CD8+, and CD45RO+ T cells was used to count the number of TILs, as described previously.[24,25]

Statistical Analysis

The significance of differences in HVEM expression according to various clinicopathologic variables was assessed using Student's *t* test, the χ^2 test, or Fisher's exact test, as appropriate. The Kaplan–Meier method was used to estimate the probability of survival, and significance was assessed using the log-rank test. Uni- and multivariate analyses were performed using the Cox proportional hazards model to identify significant prognostic predictors. All *P* values lower than 0.05 were considered statistically significant in all analyses.

RESULTS

HVEM Expression in Human CRLM and Its Relationships with Clinicopathologic Factors

We first examined the expression of HVEM in 104 surgically resected CRLM tissue samples via immunohistochemistry. We detected HVEM in the cell membrane, cytoplasm, or both in the CRLM cells. On the other hand, only limited or no HVEM expression was seen in the normal liver tissues, including normal hepatocytes, sinusoidal cells, bile duct epithelial cells, and the vascular endothelium.

To investigate the clinical importance of tumor HVEM expression in CRLM, we divided the 104 cases into a high-HVEM group ($n = 49$) and a low-HVEM group ($n = 55$) (Fig. 1A, B). Then, we examined the associations between HVEM expression and various clinicopathologic characteristics (Table 1). As a result, HVEM expression was not found to be associated with age, gender, administration of preoperative chemotherapy, tumor size, number of tumors, histologic differentiation, or tumor-sidedness of primary CRC. Regarding RAS mutation, 23.8% of the high-HVEM group had RAS mutation compared with 22.7% of the low-HVEM group ($P = 0.933$).

Impact of Tumor HVEM Expression on Postoperative Recurrence and Survival

Next, we compared postoperative recurrence and survival according to HVEM status. We found that the high-HVEM group exhibited significantly worse OS than the low-HVEM group, whereas recurrence-free survival did not differ significantly between the groups (Fig. 2A, B). The median OS time was 3.1 years in the high-HVEM group and 9.6 years in the low-HVEM group ($P = 0.002$). The 5-year postoperative survival rate was 35.8% in the high-HVEM group and 66.4% in the low-HVEM group.

Prognostic Value of Tumor HVEM Expression in CRLM

Furthermore, we examined the prognostic value of HVEM expression in CRLM. In the univariate analyses, age of 70 years or older ($P = 0.010$), presence of extrahepatic metastasis ($P = 0.023$), having five or more tumors ($P = 0.008$), a preoperative carcinoembryonic antigen (CEA) level of 20 ng/mL or higher ($P = 0.002$), a preoperative carbohydrate antigen 19-9 (CA19-9) level of 100 U/mL or higher ($P = 0.026$), a primary colorectal cancer N factor of N2–3 ($P = 0.036$), and high HVEM expression ($P = 0.002$) were identified as significant prognostic factors for OS (Table 2). In the multivariate analysis, age of 70 years or older ($P = 0.007$), having five or more tumors ($P = 0.047$), and high HVEM expression ($P = 0.002$) were identified as significant prognostic factors for OS.

Associations Between HVEM Expression in CRLM and the Recurrence Pattern or Survival After Recurrence

Recurrence was experienced by 41 patients in the high-HVEM group and 39 patients in the low-HVEM group. The initial site of recurrence in the high-HVEM group was the remnant liver alone in 17 patients (41.5%), distant sites alone in 8 patients (19.5%), and the remnant liver and distant sites in 16 patients (39.0%), whereas in the low-HVEM group, it was the remnant liver alone in 26 patients (66.6%), distant sites alone in 5 patients (12.8%), and the remnant liver and distant sites in 8 patients (20.5%). The proportion of liver-limited recurrence was significantly greater in the low-HVEM group than in the high-HVEM group ($P = 0.031$).

Repeat hepatectomy was performed for 15 (36.6%) of 41 patients in the high-HVEM group, which was a significantly fewer than in the low-HVEM group (23 of 39 patients, 59%) ($P = 0.045$). The median survival time for the patients treated with chemotherapy alone after a diagnosis of recurrence was 18.7 months in the high-HVEM group, whereas it was 44.5 months in the low-HVEM group ($P = 0.033$).

Association Between HVEM Expression and Tumor-Infiltrating T Cells

To investigate the mechanism underlying the prognostic impact of HVEM expression in CRLM, we examined the number of TILs in the CRLM using immunohistochemistry (Fig. 1D–F). We found that the number of tumor-infiltrating CD8+ and CD45RO+ T cells in the CRLM was significantly lower in the high-HVEM group than in the low-HVEM group, whereas the numbers of CD4+ T cells

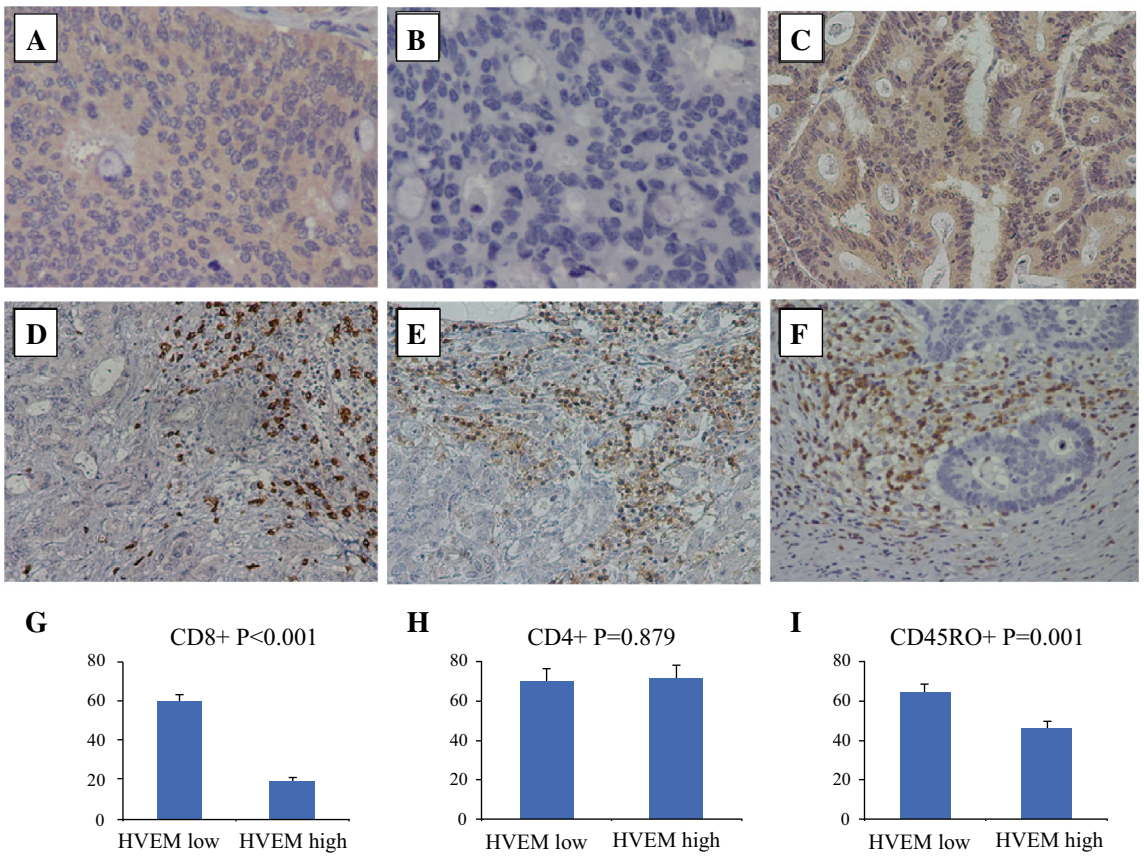


FIG. 1 **A**, **B** Representative cases of high and low herpesvirus entry mediator (HVEM) expression in surgically resected colorectal liver metastasis (CRLM). High HVEM expression was detected in **A**, and low HVEM expression was seen in **B**. **C** Representative case of high HVEM expression in surgically resected colorectal cancer (CRC), **D**–**F** representative images showing immunohistochemical staining of tumor-infiltrating lymphocytes (TILs). **D** CD8+ lymphocytes. **E** CD4+ lymphocytes. **F** CD45RO+ lymphocytes. **G–I** Relationships between tumor HVEM expression and the number of TILs in CRLM. The number of tumor-infiltrating CD8+ and CD45RO+ lymphocytes was significantly lower in the tumors that exhibited high HVEM expression ($P < 0.002$) than in those that displayed low HVEM expression ($P = 0.001$) (mean ± standard error of the mean [SEM])

in the CRLM did not differ significantly between the groups (Fig. 1G–I).

Relationship Between HVEM Expression in CRLM and Primary CRC

Finally, we examined the relationship between HVEM expression in CRLM and HVEM expression in primary CRC in the same patients. Primary CRC specimens were obtained from 59 patients. Of these, 28 primary CRC (47.5%) exhibited high HVEM expression, whereas 31 (52.5%) displayed low HVEM expression (Fig. 1C). The concordance of HVEM expression in the primary CRC and liver metastasis among synchronous cancers was 77.8% (28/36) compared with 43.5% (10/23) for metachronous cancers ($P = 0.007$) (Fig. 3A). High HVEM expression was observed in the primary CRC in 20 patients (55.6%) with synchronous CRLM and 8 patients (22.2%) with metachronous CRLM ($P = 0.037$). Among these cases, high HVEM expression also was detected in the CRLM in 16 patients (80%) with synchronous CRLM and 3 patients (37.5%) with metachronous CRLM ($P = 0.044$). The prognosis of the patients whose high HVEM expression was detected in both the CRLM and primary CRC was significantly worse than that of the patients in whom both the CRLM and CRC displayed low HVEM expression ($P = 0.017$) (Fig. 3B, C).

DISCUSSION

In this study, high HVEM expression was observed in 47.1% of patients with CRLM. High HVEM expression was not significantly associated with age, history of chemotherapy, tumor size, number of tumors, nodal or metastatic status, or histologic differentiation. However, in the multivariate analysis, high CRLM HVEM expression, age of 70 years or older, and having five or more tumors were shown to be independent poor prognostic factors for

TABLE 1 Associations between tumor herpes virus entry mediator (HVEM) expression and various clinicopathologic characteristics

	High HVEM expression (n = 49) n (%)	Low HVEM expression (n = 55) n (%)	P Value
Median age: years (range)	64 (35–82)	63 (38–82)	0.589
Gender			
Male	27 (55.1)	33 (60.0)	0.614
Female	22 (44.9)	22 (40.0)	
Timing of liver metastasis			
Synchronous	26 (53.1)	24 (43.6)	0.337
Metachronous	23 (46.9)	31 (56.4)	
Preoperative chemotherapy			
Absent	33 (67.3)	38 (69.1)	0.849
Present	16 (32.7)	17 (30.9)	
Adjuvant chemotherapy			
Absent	22 (44.9)	20 (36.4)	0.376
Present	27 (55.1)	35 (63.6)	
Extrahepatic metastasis			
Absent	41 (83.7)	41 (74.5)	0.370
Present	8 (16.3)	14 (25.5)	
Maximum tumor size (cm)			
< 5	39 (79.6)	43 (78.2)	0.860
≥ 5	10 (20.4)	12 (21.8)	
Tumor number			
< 5	36 (73.5)	46 (83.6)	0.305
≥ 5	13 (26.5)	9 (16.4)	
Preoperative CEA level (ng/mL)			
< 20	31 (63.3)	36 (65.5)	0.816
≥ 20	18 (36.7)	19 (34.5)	
Preoperative CA19-9 level (U/mL)			
< 100	42 (85.7)	48 (87.3)	0.956
≥ 100	7 (14.3)	7 (12.7)	
Location of primary colorectal cancer			
Colon	29 (59.2)	37 (67.3)	0.392
Rectum	20 (40.8)	18 (32.7)	
T factor of primary colorectal cancer (UICC 7th)			
T1–3	33 (67.3)	37 (67.3)	0.994
T4	16 (32.7)	18 (32.7)	
N factor of primary colorectal cancer (UICC 7th)			
N0–1	34 (69.4)	42 (76.4)	0.423
N2–3	15 (30.6)	13 (23.6)	
Histologic differentiation of primary colorectal cancer			
Well	18 (24.0)	28 (40.9)	0.146
Other	31 (76.0)	27 (59.0)	
Tumor location of primary colorectal cancer			
Right side	11 (40.7)	16 (59.3)	0.441
Left side	38 (49.3)	39 (50.7)	

CEA carcinoembryonic antigen, *CA19-9* carbohydrate antigen 19-9, *UICC* Union for International Cancer Control

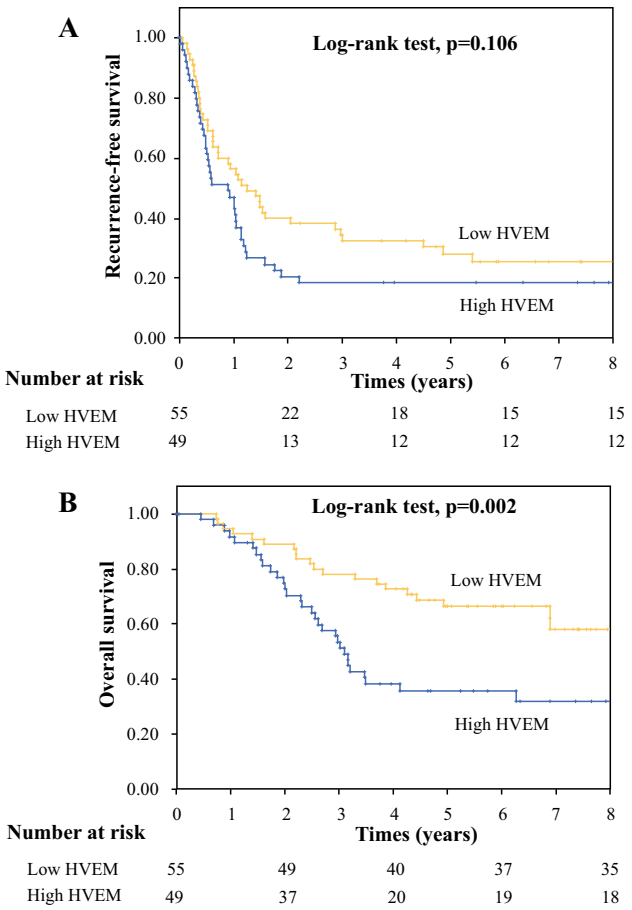


FIG. 2 Relationship between tumor herpesvirus entry mediator (HVEM) expression in colorectal liver metastasis (CRLM) and prognosis after surgical resection for CRLM. **A** Recurrence-free survival did not differ significantly between the groups (P = 0.106). **B** Overall survival was worse in the high-HVEM group than in the low-HVEM group (P = 0.002)

patients with CRLM. These findings suggest that HVEM status might play a critical role in the prognosis of CRLM independently of conventional TNM factors.

To investigate the underlying mechanism responsible for the prognostic impact of CRLM HVEM expression, we examined the number of TILs present in the CRLM via immunohistochemistry. We found that the number of tumor-infiltrating CD45RO+ and CD8+ T cells was significantly lower in the high-HVEM group than in the low-HVEM group. Considered to be memory T cells, CD45RO+ TILs can survive for many months or years and are critically important for host tumor immunity.[26,27] In addition, CD8+ TILs play an important role in the host immune defense against tumor progression in several organs.[6,28–31]

We recently reported that HVEM plays a critical role in the evasion of host antitumor immune responses in a variety of human malignancies including esophageal cancer, hepatocellular carcinoma, and primary CRC.[23–25]

TABLE 2 Uni- and multivariate analyses of factors associated with overall survival after liver resection for colorectal liver metastasis (CRLM)

		n	Univariate analysis			Multivariate analysis		
			HR	95% CI	*P* Value	HR	95% CI	*P* Value
Age (years)	< 70/≥ 70	71/33	2.09	1.19–3.64	0.010	3.21	1.38–7.52	0.007
Gender	Female/male	44/60	1.31	0.74–2.29	0.344			
Timing of liver metastasis	Synchronous/ metachronous	50/54	1.22	0.70–2.10	0.476			
Preoperative chemotherapy	Absent/present	71/33	1.68	0.96–2.96	0.069			
Adjuvant chemotherapy	Absent/present	42/62	0.58	0.33–1.00	0.051			
Extrahepatic metastasis	Absent/present	82/22	1.99	1.10–3.60	0.023	2.34	0.98–5.59	0.055
Maximum tumor size (cm)	< 5/≥ 5	91/13	0.49	0.29–1.82	0.105			
Tumor number	< 5/≥ 5	82/22	2.28	1.24–4.18	0.008	2.60	1.01–6.67	0.047
CEA (ng/mL)	< 20/≥ 20	67/37	2.35	1.36–4.06	0.002	1.55	0.70–3.41	0.281
CA19-9 (U/mL)	< 100/≥ 100	90/14	2.79	1.13–6.87	0.026	2.63	0.82–8.46	0.104
Location of primary colorectal cancer	Colon/rectum	66/38	1.07	0.61–1.89	0.809			
T factor of primary colorectal canccer (UICC 7th)	T1-3/T4	70/34	1.01	0.56–1.80	0.986			
N factor of primary colorectal cancer (UICC 7th)	N0-1/N2-3	76/28	1.86	1.04–3.34	0.036	1.87	0.74–4.70	0.186
Histologic differentiation of primary colorectal cancer	Well/other	41/63	1.44	0.81–2.57	0.211			
HVEM expression	High/low	55/49	2.34	1.34–4.10	0.002	3.35	1.41–7.93	0.006

HR hazard ratio, *CI* confidence interval, *CEA* carcinoembryonic antigen, *CA19-9* carbohydrate antigen 19-9, *UICC* Union for International Cancer Control, *HVEM* herpesvirus entry mediator

Consistent with the findings of our previous studies, the current study detected an inverse correlation between the number of TILs and tumor HVEM expression. To the best of our knowledge, this is the first study to investigate HVEM expression in CRLM.

A few investigators in previous studies have examined TILs in human CRLM. The tumor-selective activation and cytotoxic activity of CD8+ T cells in human CRLM was first reported by Wagner et al.[32] in 2008. An association between a high TIL density in CRLM and improved prognosis after liver resection was first reported by Halama et al.[9] Nakagawa et al.[10] found that low numbers of infiltrating peritumoral regulatory T cells were associated with a poor prognosis after liver resection for CRLM. Although these studies suggested that TILs in CRLM affect patients' prognosis after liver resection, the underlying mechanisms responsible for these effects are largely unknown. Our findings suggest that tumor HVEM expression might inhibit the infiltration of TILs into CRLM, and therefore might play a critical role in the prognosis of patients with CRLM.

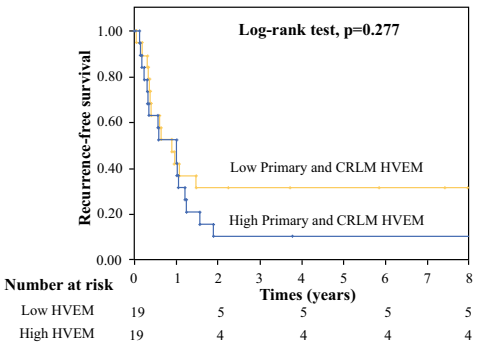
In the current study, the high-HVEM group exhibited significantly worse OS than the low-HVEM group, whereas recurrence-free survival did not differ significantly between the two groups. Although the precise mechanisms underlying these findings remain unclear, one possible explanation might be associated with the frequency of repeat hepatectomy. Some recurrent CRLM can be cured by repeat hepatectomy.[33] In fact, the frequency of repeat hepatectomy was significantly lower in the high-HVEM group than the low-HVEM group (36.6 vs. 59.0%; $P = 0.045$). Repeat hepatectomy is generally adapted for liver-limited recurrence. In this study, the proportion of liver-limited recurrence was 41.5% in the high-HVEM group compared with 66.6% in the low-HVEM group ($P = 0.031$).

Differences in tumor immunity associated with HVEM expression also might have contributed to this finding. The worse OS of the high-HVEM patients also might have been influenced by their response to chemotherapy. Halama et al.[9] reported that the reduced infiltration of immune cells into CRLM was associated with a worse response to chemotherapy. Because fewer TILs were detected in the high-HVEM group than in the low-HVEM group, the response to chemotherapy might have been unfavorable in the high-HVEM group. In fact, the survival time after recurrence in the patients treated with chemotherapy alone was significantly shorter in the high-HVEM group.

A

		Synchronous CRLM (n=36)			Metachronous CRLM (n=23)	
		CRLM			CRLM	
		High HVEM	Low HVEM		High HVEM	Low HVEM
Primary CRC	High HVEM	16	4	High HVEM	3	5
	Low HVEM	4	12	Low HVEM	8	7
		P=0.001			P=0.469	

B



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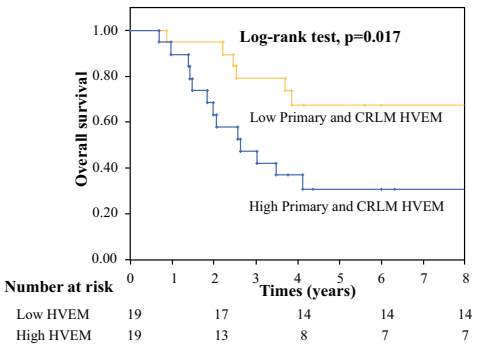

FIG. 3 **A** Relationships between tumor herpesvirus entry mediator (HVEM) expression in colorectal liver metastasis (CRLM) and primary colorectal cancer (CRC) and prognosis after surgical resection for CRLM: A high HVEM expression level in the primary CRC was significantly associated with synchronous CRLM (P = 0.001). On the other hand, the HVEM expression level of the primary CRC was not significantly associated with metachronous CRLM (P = 0.469). **B** Recurrence-free survival did not differ significantly between the patients with high CRLM and CRC HVEM expression levels and the patients with low CRLM and CRC HVEM expression levels (P = 0.277). **C** Overall survival was significantly worse for the patients with high CRLM and CRC HVEM expression levels than for the patients with low CRLM and CRC HVEM expression levels (P = 0.017)

Therefore, HVEM expression in CRLM might play a critical prognostic role through a variety of tumor immunity-related mechanisms.

Furthermore, we also examined the relationship between expression of HVEM in primary CRC and HVEM expression in CRLM. Interestingly, high HVEM expression in the primary CRC was significantly more common among the patients with synchronous CRLM than among those with metachronous CRLM. Moreover, the frequency of high CRLM HVEM expression was significantly greater among the patients with synchronous CRLM. In general, synchronous metastasis is considered to be a poor prognostic factor (compared with metachronous metastasis) for patients who undergo liver resection for CRLM.[34] The fact that synchronous CRLM is more likely to exhibit high HVEM expression might contribute to the worse prognosis.

Several studies have shown that the gene expression of metachronous CRLM differs from that of primary CRC and synchronous CRLM.[35,36] Because metachronous CRLM usually develops under long-term host immunity, the expression of HVEM in metachronous CRLM might change over time. Further fundamental studies are required to clarify the underlying mechanisms responsible for CRLM and the role of tumor HVEM expression.

This study had certain limitations. First, the number of samples evaluated for the study was relatively small. Second, the patients analyzed in this study were treated during a relatively long period. Third, the study did not include all the patients treated during this study period, mainly due to the availability of tumor samples for research purposes. Therefore, further large-scale studies are needed to verify our current findings.

In conclusion, we suggest that tumor HVEM expression might play a critical role in CRLM. Our findings indicate that not only could HVEM expression be a useful prognostic marker, but that it also might have potential as a novel immunotherapeutic target for the treatment of CRLM.

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CONFLICT OF INTEREST Yoshiyuki Sasaki, Daisuke Hokuto, Takashi Inoue, Takeo Nomi, Takahiro Yoshikawa, Yasuko Matsuo, Fumikazu Koyama, Masayuki Sho have no conflict of interest.

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